

Field Evaluation of Anthelmintic Efficacy of *Balanites egyptiaca* Against Fasciolosis in Goats

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Abstract: Fasciolosis is an endemic disease in the world and causes sever economic conditions and affecting large and small animals even the human. The present study was designed for studying the *in vivo* effects of ethanolic extract of *B.egyptiaca* on goats naturally infected with fasciolosis, through investigation of egg/gm (EPG) feces and hematological analysis. Comparison of its effects with the effects of triclabendazole (TCBZ) which is the ideal anthelmintics against fasciolosis. Twelve goats used in this study, nine were infected with fasciolosis and divided into three groups. The first group treated with ethanolic extract of *Balanites egyptiaca* (*B.egyptiaca* at dose 3 gm/kg BW), given for 3 successive days and repeated at 6th week of the experiment. The second group treated with -TCBZ at dose 10 mg/kg BW- and repeated at 6th week - of the experiment, the third group kept infected non-treated. The fourth group was non-infected non-treated. Fecal egg counts and Hematological analysis were done before and after treatment weekly for 12 weeks. The results indicated that using of ethanolic extract of *B.egyptiaca* in treatment of infected animals improved their health condition, through decreasing the number of egg of *Fasciola*/gm feces and improving the hemogram levels in the infected goats.

Key words: Fasciolosis • Sheep • *Balanites egyptiaca* • Triclabendazole

INTRODUCTION

Fascioliasis is a zoonotic disease that is caused by a trematode of species *Fasciola hepatica* (liver flukes) with about 20 million human cases around the world in its natural distribution. It is considered as emerging disease in several parts of the world, mainly in South America, Africa and Asia. It is, also, now recognized as a concern in public health due to its high pathogenicity [1, 2].

In Saudi Arabia, fascioliasis natural infection by kato thick smear and by *Fasciola*-indirect haemagglutination test (IHAT). Stool examination revealed infection in --65%-, but IHAT identified 55%- [3]. While, The overall rates of infection were 12.31%, 9.73%, 17.84% and 5.4%, respectively. Fasciolosis considered the most cause of liver condemnation and was responsible for total liver condemnation for goats as 16.9% in Taif, KSA [3, 4].

In Egypt, *Fasciola gigantica* is an important helminth parasite of livestock and is emerging as an important production and zoonotic disease. Direct and indirect losses ascribed to fasciolosis of all fluke species were estimated at 484.5 millions LE per year, according to the report of Central Organization of Mobilization and

Computation, Cairo (2000). According to some estimates every year about 600 million domestic animals become infected worldwide. In the USA alone the economical losses due to *Fasciola hepatica* (*F. hepatica*) were determined at over \$ 2 billion [5, 6].

Herbal drugs have been used since ancient times to cure diseases and several medicinal plants have been used to treat fasciolosis [8] among these *Nigella sativa*, *Caesalpinia crista*, *Saussurea lappa* [8, 9], *Allium sativum* [10] and *B.egyptiaca* [11] which have been used to treat worm infestation. No reports have been found of resistance having developed to plant anthelmintics, although many have been used in traditional veterinary medicine for very many years, or grazed or browsed as naturally occurring plants on pasture [12]. *Balanites egyptiaca*, mainly the fruit, is used by traditional healers and herbalists for treating many diseases in Africa and Asia. The fixed oil composition of fruits and oil content evaluated of its biological activity (including cytotoxicity, antiparasitic and antimicrobial activities) were performed. They found that, the oil contained 54.53% unsaturated fatty acids and 1.14% sterols. The oil exhibited anticancer activity against lung, liver and brain human carcinoma cell

lines. It also had ant-mutagenic activity against *F. gigantica* induced mutagenicity besides anthelmintic activity against hepatic worms (*S. mansoni* and *F. gigantica*) [11].

The present study was designed for studying the *in vivo* effects of ethanolic extract of *B. egyptiaca* on naturally infected sheep with fasciolosis, through investigation of egg/gm (EPG) feces and hematological analysis. Comparison of its effects with the effects of ideal drug triclabendazole, was *done*.

MATERIAL AND METHODS

Animals: Experimental animals: twelve goats used in this study. Nine were infected with fasciolosis and divided into three groups. The first group was treated with ethanolic extract of *B. egyptiaca* (3 gm/kg BW) [13], the doses of treatment given for 3 successive days and repeated at 6th week of the experiment. The second group was treated with TCBZ (10 mg/kg BW) and repeated at 6th week of the experiment, the 3rd group was kept infected non-treated. The 4th group was non-infected non-treated.

Fecal Samples: Individual fecal samples were collected directly from the rectum of each animal. The collected samples were labeled and examined for determination of EPG feces. Fecal egg counts were determined using sedimentation techniques according to Thienpont *et al.* [14] before and after treatment weekly for 12 weeks.

Blood Samples: Blood samples (each of about 2 ml) were collected from experimental animals at zero day and weekly for 12 weeks. These samples were collected via jugular vein puncture in tubes containing Ethylen Diamine Tetra Acetic Acid (EDTA) as anticoagulant, to be used for hematological investigation. The hemogram of all collected blood samples evaluated following the standard technique described by Keiser *et al.* [15]. The hemogram included the RBCs count, Hb concentration, PCV% and WBCs count. The eosinophiles count performed manually by microscopical examination of blood film stained by fields stain according to the technique described by Tankeyul *et al.* [16].

Drugs: Triclabendazole (TCBZ) “Fasinex®” was purchased from Ciba-Geigy Company.

Plants: *B. egyptiaca* fruits were purchased from Aswan Governorate, ethanolic extract of *B. egyptiaca* was prepared at medicinal and Aromatic Research Dept at National Research according to Tariq *et al.* [17].

Data Analysis: The data which were recorded during the study period were entered into Microsoft excel sheet. Data were summarized and analyzed using SPSS version 16 computer program. Data were analyzed using Epi Info version 6 statistical software [18] and for further compared using Chi-square test at critical probability of $p < 0.05$.

RESULTS AND DISCUSSION

The fecal egg count considered as the most effective tools for judging the efficacy of the fasciolosis treatment [19].

The results in Table (1) showed that the level of EPG feces showed a higher decrease in the groups treated with ethanolic extract of *B. egyptiaca* (decrease EPG feces at 3rd day 2.8 and reached to zero EPG after booster doses till the end of the experiment), followed by the groups treated with TCBZ (decrease EPG feces at 3rd day 4.3 reached to zero EPG feces after booster dose till the end week of the experiment). High efficacy of *B. egyptiaca* in sheep naturally infected with fasciolosis agreed with [20, 21] who mentioned that *B. egyptiaca* had antischistosomicidal effect. Moreover, [13, 22] observed a fasciolicidal effect of *B. egyptiaca* appeared more effective in treatment of fasciolosis than TCBZ.

Our results agreed with those of Keiser *et al.* [15] where they reported that, TCBZ is the only available drug for treatment of fasciolosis and is more efficient especially in the area endemic in fasciolosis. In addition, Daniel *et al.* [19] reported that, TCBZ causes severe decrease of *Fasciola* eggs in EPG feces examined.

The results of Table (2) showed that the most efficient treatments of fasciolosis in goats that improve the RBCs count ethanolic extract of *B. egyptiaca* (increased RBCs count from 5.5 in the 1st week till reached 7.4 at the end of the experiment). The TCBZ also improve the RBCs count (level of RBCs count increased from 5.5 at 1st week until reached 6.4 at 12 week. This results agreed with those of Ezzat and Aboul-Ela and Matanovic *et al.* [23 and 24], where they reported that, sheep infected with fasciolosis suffering from severe decrease in RBCs level than the flock free from infection. In addition, these results agreed with Kok *et al.* [13] who mentioned that *B. egyptiaca* treated goats showed a slight progress in RBCs count.

The results of TCBZ attributed to the TCBZ induced elimination of flukes and healing the hepatic lesions. These results agreed with those of Matanovic *et al.* [24] TCBZ administration induced the elimination of flukes and healing of the majority of hepatic lesions but did not

Table 1: Effect of different treatments on EPG feces at different weeks of experiment:

Groups	Weeks													
	Do	D3	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
I Treated with Alco extract of <i>B.egyptiaca</i>	Ba5.1±0.0	Cb2.8±0.3	Cc1.2±0.3	Bc1±0.5	Cd0.83±0.3	Ce0.5±0.1	Ce0.5±0.1	De0.50±0.1	Cf-Ve	Cf-Ve	Cf-Ve	Cf-Ve	Cf-Ve	Cf-Ve
II Treated with TCBZ	Aa6.7±0.6	Bb4.3±1.2	Cc1.2±0.3	Bc1±0.5	Cd0.83±0.3	Cd0.17±0.3	Cd0.5±0.0	Dd0.5±0.01	Ce-Ve	Ce-Ve	Ce-Ve	Ce-Ve	Ce-Ve	Ce-Ve
III C Control infected non treated	Ab6.7±1.5	Ab6.3±0.6	Ab6±1.0	Ab6.3±0.6	Ac5.17±1.3	Ac5.67±1.2	Ac5.5±1.3	Ab6.67±0.6	Ab6.67±1.50	Ab6.67±0.60	Ab7±1.0	Aa7.33±0.80	Aa7.50±1.0	Aa7.30±0.50

Capital - letters Means within the same column of different letters are significantly different at (P < 0.01).
Small - letters: Means within the same raw of different letters are significantly different at (P < 0.01)
D=day W=week G= groups
TCBZ Triclabendazole
B.egyptiaca *Balanites egyptiaca*
EPG Egg per gram
SD Standard deviation

Table 2: Effect of different treatments on RBCs (10⁶/μl) and WBCs(10³/μl)count at different weeks of experiment.

Weeks	RBCs (10 ⁶ /μl)				WBCs(10 ³ /μl)			
	I Treated with Alc extract of <i>B.egyptiaca</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non- infected non-treated	I Treated with Alc extract of <i>B.egyptiaca</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non- infected non-treated
D0	Cc5.5±0.5	Bc5.5±0.5	Bc5.4±0.5	Ba9.97±0.7	Ad19.2±0.3	Ae18.6±1.7	Ib29.10±0.4	Af13.9±1.5
W1	Cc5.6±0.1	Bc5.6±0.3	Ac5.8±0.4	Ba9.97±0.6	De13.5±0.5	Cd14.4±0.4	Ib29.4±0.5	Be13.0±1.3
W2	Bc6.3±0.2	Bd5.7±0.2	Ad5.8±0.4	Ba9.8±0.3	Cd14.4±0.4	De13.1±0.2	Ba35.4±1.3	Vf11.9±0.4
W3	Bc6.7±0.1	ABd5.9±0.1	Ad5.9±0.4	Aa10.0±0.4	Bd15.7±2.7	De12.91±0.2	Aa40.2±1.5	Df11.3±0.4
W4	ABc6.9±0.1	Ac6.2±0.2	Be5.1±0.1	ABa9.9±0.4	Cc14.8±1.3	Ce14.0±0.3	Fa32.0±1.3	Cd11.8±0.9
W5	Ac7.1±0.1	Ad6.3±0.2	Be5.1±0.2	Aa10.1±0.2	Cd14.2±1.0	Cd14.6±2.10	Hb30.97±0.2	Ce11.9±1.0
W6	Ac7.3±0.2	Ad6.4±0.2	Be5.2±0.2	Aa10.2±0.3	Cd14.3±1.0	Cd14.6±2.1	Gb31.4±0.5	Ce12±1.0
W7	Ac7.2±0.2	Ad6.1±0.5	Be5.2±0.2	Aa10.3±0.3	Cd11.7±0.1	Bd15.10±0.3	Ga31.6±1.5	CDe11.6±1.2
W8	Ab7.3±0.2	Ad6.2±0.4	Be5.2±0.2	Aa10.2±0.4	Ee11.5±0.1	Bd15.0±0.2	Fa32.0±1.0	CDe11.7±0.6
W9	Ac7.9±0.2	Ad6.3±0.5	Be5.4±0.2	Aa10.1±0.3	Ee11.5±0.1	Bd15.0±0.2	Ea33.0±1.0	CDe11.7±0.6
W10	Ac7.3±0.2	Ad6.2±0.4	Be5.4±0.1	Aa10.1±0.3	Ee11.5±0.2	Bd15.10±0.2	Da34.7±1.0	De11.1±0.8
W11	Ac7.3±0.7	Ad6.3±0.2	Be5.3±0.1	Aa10.3±0.3	Ed11.7±0.2	Cc14.7±0.4	Cb35.2±1.4	Dd11.2±0.3
W12	Ab7.4±0.8	Ac6.4±0.2	Bd5.3±0.2	Aa10.2±0.4	Ed11.6±0.4	Cc14.0±0.1	Db34.6±2.1	CdD11.4±0.2

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uD=day W=week G= groups
RBCS Red blood cells
WBCs White blood cells

Table 3: Effect of different treatments on Hb conc (g/dl) and PCV% at different weeks of experiment

Weeks	Hb conc (g/dl)				PCV%			
	I Treated with Alc extract of <i>B.egyptiaca</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non- infected non-treated	I Treated with Alc extract of <i>B.egyptiaca</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non- infected non-treated
D0	De7.5±0.4	Cd7.9±0.2	Bd7.2±1.5	Aa12.5±0.6	Ge18.4±0.5	Fd17.9±0.9	Ce16.1±0.9	Ba29.8±2.1
W1	Dc7.7±0.1	Cc8.2±0.6	Ac7.9±0.1	Aa12.3±0.6	Fe19.2±0.9	EFD18.1±0.7	BCe16.2±0.9	Ca28.8±0.6
W2	Cc8.2±0.3	Cc8.2±0.5	Bd7.2±0.3	ABa11.7±0.7	Ec20.7±0.5	Rd18.3±0.4	Bf16.6±0.4	Ca28.8±0.8
W3	BCc8.6±0.1	Cc8.2±0.4	Ad7.9±0.9	Ba11.1±0.4	De22.1±0.5	Ed18.5±0.4	Be16.8±0.6	Da28.4±0.7
W4	Be8.8±0.1	Cc8.3±0.4	ABd7.5±0.7	Ba11.5±0.8	Ccj23.3±9.7	Dd19.2±0.4	Ae17.3±0.7	Ca28.9±0.3
W5	Be9.1±0.2	Bd9.0±0.2	Ae7.8±1.1	ABa11.7±0.4	Ac25.1±0.8	Dd19.8±0.3	Bf16.7±1.4	Ca29.2±0.4
W6	Be9.2±0.1	Be9.1±0.2	Be7.2±1.5	ABa11.8±0.4	Ac25.1±0.8	Cd20.2±0.7	Bf16.6±1.5	Ca29.2±1.3
W7	Be8.8±1.3	Be8.9±0.4	ABd7.6±1.0	ABa11.8±0.5	Ac25.1±1.5	Cd20.0±1.1	Ae17.11±1.7	Ba29.7±0.7
W8	Bd8.7±1.4	Be9.0±0.3	Be7.2±1.7	ABa11.9±0.4	Ac25.1±1.4	Bd23.5±0.5	Bf16.6±1.6	Ba29.6±0.7
W9	Bd8.9±1.3	ABc9.2±0.4	ABe7.4±1.6	ABa11.9±0.4	Ac25.1±1.4	Bd23.7±0.9	Ae17.1±1.3	Ba29.7±0.7
W10	Be9.1±1.4	Be9.1±0.3	Be7.3±0.2	Aa12±0.4	Ab25.3±1.5	Be23.5±0.6	Ad17.1±0.8	Ba29.7±0.6
W11	Ab9.8±0.7	Ab9.4±0.1	Bd7±2.4	Aa12.1±0.4	Bc23.8±2.4	Ab24.3±0.3	Ad17.3±2.4	Aa30.00±0.3
W12	Ab9.9±0.8	Ab9.7±0.3	Bd7.3±1.5	Aa12.2±0.5	Bc23.8±2.3	Ab24.8±0.3	Ad17.1±1.0	Aa30.7±0.8

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W= week
Hb Hemoglobin
PCV Packed cell volume

Table 4: Effect of different treatments on eosinophile levels ($10^3/\mu\text{l}$) at different weeks of experiment.

Weeks	eosinophile ($10^3/\mu\text{l}$)			
	I Treated with A/c extract of <i>B.egyptiaca</i>	II Treated with TCBZ	III Control infected non-treated	IV Control non-infected non-treated
D0	Ab0.81±0.01	Ab0.88±0.02	Aa1.4±0.02	Ac0.4±0.1
W1	Bb0.6±0.03	Bb0.54±0.03	Aa1.4±0.1	Ab0.4±0.1
W2	Bb0.6±0.03	Cb0.41±0.02	Aa1.4±0.1	Ab0.4±0.1
W3	Bb0.6±0.1	Cb0.4±0.01	Aa1.5±0.1	Ab0.4±0.1
W4	Bb0.5±0.1	Cb0.4±0.03	Aa1.5±0.06	Ab0.4±0.1
W5	Bb0.5±0.05	Cb0.4±0.02	Aa1.4±0.1	Bb0.33±0.1
W6	Dc0.44±0.04	Cb0.4±0.02	Aa1.5±0.1	Bc0.34±0.04
W7	Dd0.32±0.02	Cc0.4±0.01	Aa1.4±0.03	Ad0.32±0.02
W8	Dd0.32±0.02	Cd0.39±0.01	Aa1.4±0.03	Ad0.4±0.05
W9	Dd0.35±0.02	Cc0.42±0.04	Aa1.5±0.06	Ac0.4±0.04
W10	Dd0.35±0.01	Cc0.43±0.03	Aa1.4±0.5	Ac0.4±0.04
W11	CDb0.4±0.03	Cb0.46±0.06	Aa1.3±0.09	Ab0.43±0.04
W12	CDb0.38±0.02	Cb0.44±0.07	Aa1.3±0.01	Ab0.44±0.01

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Small - letters: Means within the same raw of different letters are significantly different at ($P < 0.01$).

prevent severe hepatic damage produced by later infections. This will improve the body conditions of the sheep and regeneration of RBCs.

The results indicated that, the groups that treated with extract of *B. egyptiaca* showed gradual decrease in WBCs count from 19.2 at zero day till reached 11.6 at the end of the experiment) and TCBZ showed a lower level of WBCs (decreased WBCs count from 18.6 at zero day till reached 14.0 at the end of the experiment). The results also indicated that the control infected non-treated group of higher WBCs level (ranged from 29.1 to 40.2 all over the experiment) than the control non-infected non-treated group (ranged from 11.1 to 13.9 all over the experiment).

These results agreed with those of Matanovic *et al.* [24], who reported that, sheep infected with fasciolosis suffering from increasing of WBCs count than the flock free from infection.

Our results indicated that, the most efficient treatments on Hb conc were ethanolic extract of *B. egyptiaca* as it improve the Hb conc (increased Hb conc from 7.5 at zero day till reached 9.9 at the end of the experiment), while TCBZ treated group (increase Hb conc from 7.9 at zero day till reached 9.7 at the end of the experiment). These results agreed with those of Matanovic *et al.* [24], where they reported that, sheep infected with fasciolosis suffering from severe decrease in Hb level than the flock free from infection. In addition, Koko *et al.* [13] reported that goats infected with fasciolosis and treated with *B. egyptiaca* showed a slight progress in Hb conc, Zaoui *et al.* [22].

The results of TCBZ attributed to the TCBZ induced elimination of flukes and healing the hepatic lesions thus results agreed with those of Matanovic *et al.* [24] TCBZ

administration induced the elimination of flukes and healing of the majority of hepatic lesions but did not prevent severe hepatic damage produced by later infections. This will improve the body conditions of the sheep and regeneration of RBCs and improve the Hb%.

The results of PCV% at different weeks, showed that, the most efficient treatments on PCV% were ethanolic extract of *B. egyptiaca* as it improve the PCV% (increased from 18.4 at zero day till reach 23.8 at the end of the experiment), TCBZ treated group (increase from 17.9 till reached 24.8 at week 12 of the experiment).

These results agreed with those of Matanovic *et al.* [24], where they reported that, sheep infected with fasciolosis of low PCV% than the flock free from infection. Koko *et al.* [13] mentioned that goats infected with fasciolosis and treated with *B. egyptiaca* showed a slight progress in PCV%.

The results showed decreased the eosinophile level in ethanolic extract of *B. egyptiaca* (decrease 0.88 at zero day till reached 0.44 at the end of the experiment) compared to TCBZ treated group. The result also, indicated that the control infected non-treated group of higher eosinophile level than the sheep free from infection (Control non-infected non-treated). This results agreed with those of Matanovic *et al.* [21] and Zaoui *et al.* [22], where they reported that, sheep infected with fasciolosis showed a higher level of eosinophile level than the flock free from infection.

The beneficial effect of ethanolic extract of *B. egyptiaca* against fasciolosis and the improvement of the sheep conditions with improvement of decreasing and inhibition egg number/gm, WBCs, eosinophile and increasing RBCs, Hb and PCV.

Also, our results exactly similar to results of [24] where they observed that, *B. egyptiaca* (L) Del. (Balanitaceae) fruit mesocarp water extract (traditionally used as an anthelmintic in the Sudan) causes the characteristic lesions of liver fasciolosis, egg/gm of feces (EPG), packed cell volume (PCV), hemoglobin concentration, total red blood cells count (RBC), total white blood cells count (WBC) and eosinophile% were improved and significantly different from control and treated groups ($P < 0.05$).

Results of TCBZ agreed with those of Bashir *et al.* [20] on Egyptian sheep, who mentioned that there is no any fasciolosis signs and no eggs of *Fasciola* with improvement of hematological parameters of the treated sheep.

CONCLUSION

Fasciolosis considered the most cause of liver condemnation and was responsible for total liver condemnation for goats as 16.9%. Studing the effects of plant extracts *B. egyptiaca* and TCBZ on adult *F. gigantica*, through hematological examination revealed that, decreasing the number of egg of *Fasciola*/gm feces and the blood parameter returned to its normal levels, leading to improved goats health condition.

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